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Behavioural Brain Research 118 (2001) 107–110

BEHAVIOURAL BRAIN RESEARCH

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Short communication

Effects of the 5-HT_6 receptor antagonist Ro 04-6790 on learning consolidation

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Received 27 June 2000; received in revised form 21 August 2000; accepted 21 August 2000

Abstract

The 5-HT_6 receptor antagonist Ro-04-6790 or 8-OH-DPAT injection improved learning consolidation on an autoshaping task, while mCPP, scopolamine and dizocilpine decreased the performance. The effect induced by scopolamine, but not that induced by mCPP, was reversed completely by Ro-04-6790, while dizocilpine effect was antagonized partially. Nevertheless, ritanserin or WAY 100635, but not Ro 04-6790, antagonized the 8-OH-DPAT facilitatory effects on learning consolidation. As WAY 100635 did not modify the Ro 04-6790 facilitatory effect, hence 5-HT_{1A}, and/or 5-HT_7, but not 5-HT_6, receptors might mediate the 8-OH-DPAT facilitatory effect on learning consolidation. Since, the Ro 04-6790 facilitatory effect was unaffected by 5-HT_{1A}, 5-HT_{2A}/$_{2B}$/$_{2C}$, 5-HT_3 or 5-HT_4 receptor blockade, thereby, the facilitatory effect induced by Ro 04-6790 involved specifically 5-HT_6 receptors. Indeed, the present data provide further support to the notion that, 5-HT_6 receptors play a significant part in the learning consolidation under normal and dysfunctional memory conditions.

Keywords: Serotonin; Receptors; 5-HT; Learning; Autoshaping; Rat

1. Introduction

Serotonergic neurotransmission involves multiple 5-hydroxytryptamine (5-HT) receptor subtypes (5-HT_1 to 5-HT_7) [1]. Among these, the 5-HT_6 and 5-HT_7 receptors show a regional distribution within the central nervous system [1,4,8], in areas, which have been associated with learning and memory processes (see [13,14], for reviews). Diverse physiological and/or behavioral effects following manipulation of 5-HT_6 receptors have been reported [1,4,9]. For instance, 5-HT_6 antisense oligonucleotide decreased 5-HT_6 gene expression and the induced behaviors by antisense treatment were antagonized by atropine (a muscarinic antagonist) [3]. Rats 5-HT_6 antisense treatment produced an enhanced spatial learning acquisition in the water maze [2]. It should be noticed that, the studies mentioned above were based upon the sole use of nonselective either, agonist or antagonist drugs for 5-HT_6 receptor. Nevertheless, a growing interest for the 5-HT_6 receptors as target by the drugs development useful for psychiatric disorders treatment [9], such as schizophrenia. Interestingly, an association between the 5-HT_6 receptor polymorphism C267T and Alzheimer's disease (AD) has been reported [20]. Indeed, two novel, presenilin 1 (PS1) and presenilin 2 (PS2) have been implicated casually in the pathogenesis of AD and more than 50 misense mutations of PS1 are known [5], including the polymorphism C267T. Accordingly, drugs acting at 5-HT_6 receptors could modulate learning and memory. Hence, in the current work, it was decided to study the effect of the 5-HT_6 antagonist Ro 04-6790 [4], the 5-HT_{1A}/$_7$ agonist 8-OH-DPAT, the 5-HT_{1A} antagonist WAY100635, the 5-HT_{1A}/$_{1B}$/$_{1D}$/$_{2A}$/$_{2C}$/$_7$ agonist/antagonist mCPP, the 5-HT_{2A} antagonist ketanserin, the 5-HT_{2A}/$_{2C}$/$_6$/$_7$ antagonist ritanserin, the 5-HT_3 antagonist ondansetron, the 5-HT_4 antagonist GR125487 [1,10], scopolamine (an anticholinergic), dizocilpine (an

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PII: S0166-4328(00)00316-8

NMDA antagonist) and phenserine (an AChE inhibitor) [13] on associative learning. An autoshaping test was used here, which had shown to be useful to study learning and memory changes produced by age and/or drugs (see [13,18]). Importantly, whether drugs are administered before training, results very difficult to determine whether these act on memory or other processes (e.g. attention, motivation, locomotor activity, etc.; [13]; for recent review, see [11]); however, the post-learning treatment strategy allows to exclude most of the problems that the former strategy presents, i.e. producing changes on attention, motivation, locomotor activity, etc.; unrelated with learning and memory per se.

2. Methods

Adult male Wistar rats were housed collectively in a temperature- and light-controlled room under a 12-:12-h light-dark cycle (light on at 7:00 h). Water and food were provided ad libitum for a week. After that period, body weights were reduced to 85% by gradually reducing the food intake during 7 days.

2.1. Autoshaping training

Each rat was placed in an experimental chamber and allowed to habituate to the experimental environment until the animal found and ate 50 food pellets (each pellet 45 mg). Immediately afterwards, the program began. This consisted in the presentation of a retractable illuminated lever for 8 s (conditioned stimulus, CS), followed by delivery of a food-pellet (unconditioned stimulus, US) every 60 s. When the animal pressed the CS, the lever was retracted, the light was turned off, and a food pellet (US) was delivered immediately; this was defined as a conditioned response (CR). The increase or decrease in percentage of CR was considered as an enhancement or impairment in learning, respectively. The first session consisted of 10 trials and the second session of 20. All compounds were injected immediately following the first autoshaping session; then, the sessions test was performed 24 h later and, in other groups, the animals were given Ro 04-6790 immediately after the first autoshaping session and, 10 min later, the animals received scopolamine or dizocilpine and 24 h later the session test. The data displayed correspond to the session test (i.e. second session) [13].

2.2. Drugs

The drugs used in the present study were: Ro 04-6790 (4-amino-*N*-[2,6-bis(methylamino)-4-pyrimidinyl]-benzenesulfonamide dihydrochloride) (RBI-Sigma, Saint Louis, MO, USA); scopolamine HBr, dizocilpine maleate, 8-OH-DPAT ((±)-8-hydroxy-2-(di-n-propilamino) tetralin HCl), WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanene carboxamide 6trihydrochloride), mCPP (1-(3-chlorophenyl)piperazine dihydrochloride), ketanserin tartrate, and ritanserin (Research Biochemical Inc., Wayland, MA, USA); ondansetron and GR125487 (Glaxo-Wellcome, UK), and phenserine (Gerontology Research Center, NIH, Baltimore, MA, USA). All drugs were injected intraperitoneally (i.p.) and dissolved in saline solution or methylcellulose in a volume of 1 ml/kg. It should be noticed that, all drugs used here, but Ro 04-6790, have their respective full dose–response curves reported previously [13–16].

Operant chambers (Coulbourn Instruments, Lehigh Valley, PA, USA) for rats with standard sound-attenuation were used. Chambers were 25-cm wide, 29-cm long, and 25-cm high. A retractable lever was mounted 4 cm above the floor and 10 cm from the right and left walls. The lever required 10 g F to operate. A food magazine for rat pellets (Bio Serv, Frenchtown, NJ, USA) was located 5 cm to the right of the lever and 3 cm above the floor. A house light was located in the right top corner. Solid-state programming equipment was used for control and recording (Coulbourn Instruments, Lehigh Valley, PA, USA).

2.3. Data acquisition and statistical analysis

As in previous works [13–18], the responses (CR) in the CS presence were divided by the trials in session, expressed as a percentage of the total trials, and analyzed using analysis of variance (ANOVA) followed by additional post-hoc comparisons using the Tukey test. In all comparisons, $P < 0.05$ was used as criterion for significance. The n was eight per group, and animals were used only once.

3. Results

As depicted in Table 1, the Ro 04-6790 administration increased significantly the CR% [$F(4,39) = 6.2$; $P < 0.05$], and this effect was significant at the 5 mg/kg dose. As reported previously [16], 8-OH-DPAT, significantly [$F(4,39) = 3.5$; $P < 0.05$] increased the CR%. At the tested doses, neither WAY100635, ketanserin, ritanserin, ondansetron, nor GR125487 by itself, affected the CR percentage of mCPP [$F(6,55) = 1.0$; $P > 0.05$], [$F(6,55) = 1.3$; $P > 0.05$]. In contrast, scopolamine [$F(3,31) = 3.9$; $P < 0.05$] or dizocilpine [$F(3,31) = 4.9$; $P < 0.05$], but not phenserine[$F(3,31) = 1.2$; $P > 0.05$] administration decreased significantly the CR% (Table 1). Nevertheless, Ro 04-6790 blocked the scopolamine or dizocilpine impairment-effect, but not that induced

Table 1
Effects of various 5-HT receptor drugs on the percentage of conditioned response (CR) in an autoshaping learning task

Treatment (mg/kg)	CR (%)
Control	10 ± 2
Ro 04-6790 (1)	12 ± 5
Ro 04-6790 (5)	26 ± 7[a]
Ro 04-6790 (10)	29 ± 10
8-OH-DPAT (0.062)	29 ± 5[a]
Control	10 ± 2
WAY 100635 (0.01)	11 ± 3
mCPP (5)	2 ± 2[a]
Ketanserin (0.001)	16 ± 3
Ritanserin (0.1)	17 ± 4
Ondansetron (0.01)	17 ± 5
GR 125487 (0.78)	9 ± 4
Control	12 ± 3
Scopolamine (0.17)	3 ± 1[a]
Dizocilpine (0.1)	2 ± 1[a]
Phenserine (0.5)	19 ± 6

[a] Values are significantly different from control-vehicle ($P<0.05$ by Tukey test).

by mCPP (Table 2). Moreover, WAY 100635, ketanserin, ondansetron or GR 125487 had no effect by itself and did not modify Ro 046790 effect (Table 2). Ritanserin had no effect by itself, however, significantly [$F(4,39)=3.9$; $P<0.05$] antagonized the increase of CR% induced by Ro 04-6790 (Table 2). As previously reported [16] the 8-OH-DPAT facilitatory effect was antagonized by WAY 100635 and ritanserin [17,18], but not significantly affected Ro 04-6790 (the current work).

Table 2
Effects of various 5-HT receptor drugs on the percentage of conditioned response (CR) in an autoshaping learning task[a]

Treatment (mg/kg)	CR (%)
Control	10 ± 2
Ro 04-6790 (5)	26 ± 7*
Ro 04-6790 (5) + 8-OH-DPAT (0.062)	16 ± 5+
Ro 04-6790 (5) + WAY 100635 (0.01)	31 ± 3*
Ro 04-6790 (5) + Ketanserin (0.001)	28 ± 3**
Ro 04-6790 (5) + Ritanserin (0.1)	17 ± 3+
Ro 04-6790 (5) + Ondansetron (0.01)	27 ± 5*
Ro 04-6790 (5) + GR 125487 (0.78)	31 ± 4*
Control	11 ± 3
WAY 100635 (0.1) + 8-OH-DPAT (0.062)	16 ± 5+
Ro 04-6790 (5) + mCPP (5)	6 ± 2+
Ro 04-6790 (5) + Scopolamine (0.17)	16 ± 3+
Ro 04-6790 (5) + Dizocilpine (0.1)	9 ± 5*

[a] *, Values are significantly different from the control-vehicle or, +, 5-HT antagonist control group ($P<0.05$ by Tukey test).

4. Discussion

Inasmuch as, in the present study, the animals received the drugs after the first training session and once they had the opportunity to learn where to find food-pellets, and thus excluding nonspecific change [11]. Therefore, these data reflect an effect in the learning consolidation [11,13,14]. The major finding of the present study was that, post-training injection of the 5-HT_6 receptor antagonist Ro 04-6790 alone enhanced learning consolidation. While, ritanserin or WAY 100635 had no effect; the 8-OH-DPAT facilitatory effect was nevertheless, completely or partially (but not significantly) antagonized by WAY 100635 or Ro 04-6790, respectively, suggesting that 5-HT_{1A}, 5-HT_6 and 5-HT_7 receptors could not interacting during learning consolidation. The Ro 04-6790 facilitatory effect on learning consolidation was unaffected by WAY 100635 (a 5-HT_{1A} antagonist), ketanserin (a 5-HT_{2A-2c} antagonist), ondansetron (a 5-HT_3 antagonist), or GR 125487 (a 5-HT_4 antagonist), but reversed by ritanserin (a $5\text{-HT}_{2A-2C/6/7}$ antagonist) [1,4]. Hence, it seems logical to conclude that, 5-HT_6 receptors are specifically mediating the Ro 04-6790 facilitatory effects. Moreover, 5-HT_6 receptor blockade did not affect the mCPP (a $5\text{-HT}_{1A/1B/1D/2A/2C/7}$ agonist/antagonist) impairment-induced effect, nevertheless, Ro 04-6790 completely (scopolamine) or partially (dizocilpine) normalized a poor memory. Moreover, AChE inhibition modified weakly the Ro 04-6790 facilitatory effect, suggesting that 5-HT_6 receptor may be affecting the cholinergic neurotransmission directly. Operational (i.e. pharmacological) information [1,13,14,19] regarding 5-HT_{1A} and/or 5-HT_7 receptors supports the conclusion that these receptors manipulation did not alter significantly the Ro 04-6790 facilitatory effects on learning consolidation.

The present findings are consistent with emerging evidence indicating that 5-HT_6 receptors participation on cognitive processes, particularly in learning and memory (see [4], for review). For example, central administration of antisense oligonucleotides targeted for the 5-HT_6 receptors had no effect in visual acuity or swim speed in rats nonetheless, they produced a facilitated performance in the water maze [2]. Similarly, the highly brain penetrant 5-HT_6 receptor antagonist SB-271046 [19] improved retention in the water maze and, produced a significant performance improvement of aged rats in an operant-delayed alternation task. In this connection, it is noteworthy that, systemic administration of other 5-HT_6 receptor antagonists, SB-271046, produced a significant tetrodotoxin-dependent, increase in extracellular levels of glutamate and aspartate within the frontal cortex [6]. More importantly, 5-HT_6 receptors occur in hippocampus, amygdala, and several cortical layers [1,4], brain areas involved in learning and

memory [13]. And the 5-HT_6 267C allele gene has been reported as a risk factor for Alzheimer's disease [20]. Altogether, this information provides additional support to physiological, pathophysiological and therapeutic roles of 5-HT systems in learning and memory (see [13,14], for reviews).

Cognitive function, particularly learning and memory, is impaired markedly in most schizophrenics patients and atypical antipsychotics drugs pharmacologically related to clozapine and 5-HT_{2A} receptor antagonism, may improve cognitive dysfunction [12]. Indeed, in support to this contention, it has been suggested [17,18] that 5-HT_{2A}/$_{2B}$/$_{2C}$ receptors blockade may provide some benefit to reverse poor learning consolidation conditions associated with serotonergic, cholinergic and/or glutamatergic neurotransmission, such as those found in AD patients, as well as target for novel antipsychotics. However, clozapine is a high-affinity antagonist at both human and rat $5HT_6$ receptors [4], and considering the present findings and the above-mentioned evidence, it could hence reasonable to not exclude either 5-HT_{2A} and 5-HT_6 receptors potential involvement in clozapine-like drugs in learning and memory studies. Of course, future studies must also explore the effects of agents such as the 5-HT_6 receptor agonist 2-ethyl-5-methoxy-*N*, *N*-dimethyltryptamine and its 2-ethyl substituent with a phenyl group, which retains 5-HT_6 receptor affinity but lacks agonist character [7]. The latter compound could represent a 5-HT_6 receptor inverse agonist.

Acknowledgements

The author thanks the pharmaceutical companies for the generous gifts (see Section 2.2). The author wants to thank Sofia Meneses for the review of the language. The author appreciates greatly the technical assistance of R. Gonzdlez and H. Vazquez for the bibliographic assistance. Supported by CONACYT grant 28398M.

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